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A NEW COUMARIN FROM *CLAUSENA EXCAVATA*

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Abstract – Chemical investigation of the fruits and stems of *Clausena excavata* led to the isolation and identification of a new coumarin namely clausenaexcavin (**1**) along with five known compounds (**2**-**6**). All structures were characterized by extensive 1D and 2D NMR spectroscopic methods.

Clausena excavata is known locally as “Sun-soak” belonging to Rutaceae. Several parts of this plant have been used for the treatment of cold, malaria, AIDS, dermatopathy, abdominal pain, snake-bite, and as a detoxification agent.[1] A number of coumarins and alkaloids have been reported from several parts of this plant.[1] Some of these compounds showed anti HIV-1,[1] antibacterial,[2] antiplasmodial,[3] anticancer,[4] antimycobacterial,[5] and antifungal activity.[5] As part of our continuing chemical studies on Thai medicinal plants, we now report herein the isolation and identification of a novel coumarin along with five known compounds which were isolated from the fruits and stems of *C. excavata* collected from Satoon Province, southern part of Thailand.

The crude extracts from the fruits and stems of *C. excavata* were subjected to a succession of chromatographic procedures afforded a new coumarin namely clausenaexcavin (**1**) together with five known compounds (**2**-**6**) (Figure 1). The structure of a new compound was characterized by spectroscopic methods including UV, IR, 1D- and 2D- NMR and HRMS.

Clausenaexcavin (**1**) was isolated as colorless viscous oil with a molecular formula $C_{19}H_{24}O_7$ on the basis of the $[M\text{-}H_2O]^+$ ion at *m/z* 346.1422 in the HREIMS (calcd *m/z* 346.1416). The UV spectrum showed maxima absorption bands at 207, 230, 258 and 318 nm indicating conjugated system in the molecule whereas the IR spectrum showed the hydroxyl and carbonyl functionalities at 3408 and 1718 cm^{-1} respectively. The ^1H NMR signals at δ 6.25 (H-3) and 7.60 (H-4) (each d, J = 9.6 Hz) and 6.93 (H-5) and 6.80 (H-6) (each d, J = 8.5 Hz) indicated the presence of 7,8-dioxygenated coumarin nucleus.[6] In addition the existence of 2,3,7-trihydroxy-3,7-dimethyloct-5-enyloxy group was also observed in the ^1H NMR spectrum at δ 5.77 (d, J = 16.0 Hz, H-6′), 5.73 (m, H-5′), 4.99 (dd, J = 3.0, 11.5 Hz, H-1′a), 4.09 (dd, J = 9.0, 11.5 Hz, H-1′b), 3.98 (dd, J = 3.0, 9.0 Hz, H-2′), 2.44 (dd, J = 6.0, 14.0 Hz, H-4′a), 2.29 (dd, J = 7.5, 14.0 Hz, H-4′b), 1.34 (s, H-9′), 1.30 (s, H-8′) and 1.29 (s, H-10′). The COSY and HMBC correlations (Figure 2) were also supported this moiety. The HMBC correlations between H-1′, H-5 and H-6 and C-7 (146.4) indicated the side chain moiety was located at C-7 of coumarin framework. The geometry of double bond at C-5′/C-6′ was identified to be *E*-geometry due to the large amount of J value of H-6′ (16.0 Hz). Therefore, clausenaexcavin was identified to be **1**.

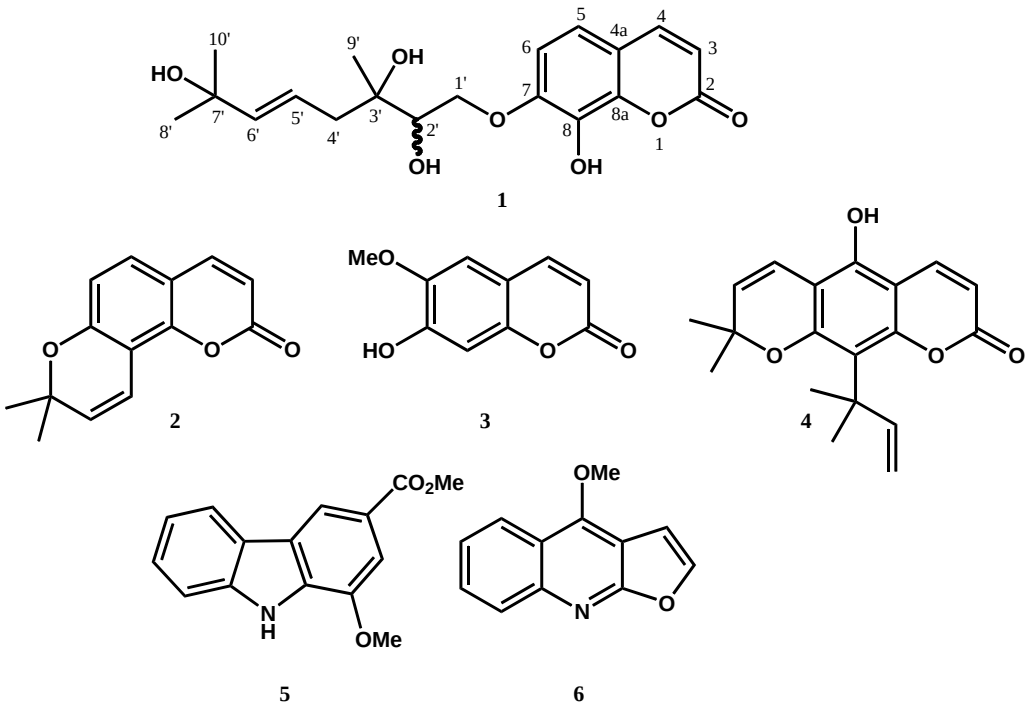

Figure 1. Compounds isolated from *C. excavata*

The remaining known compounds were identified as seselin (**2**),[7] scopoletin (**3**),[8] 5-hydroxydentatin (**4**),[9] mukonine (**5**)[10] and dictamine (**6**)[11] by extensive 1D and 2D NMR spectroscopic methods and comparison with reported spectral data in literatures.

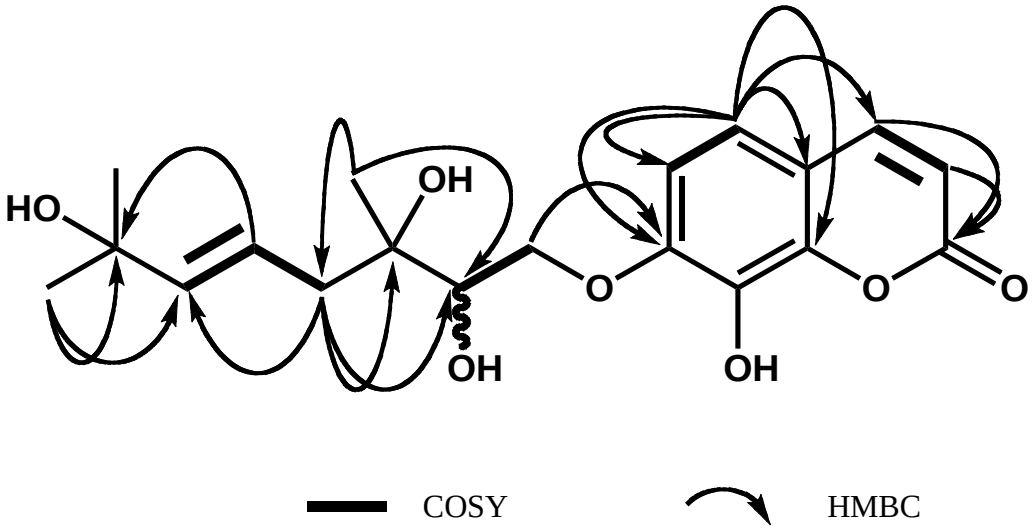

Figure 2. COSY and selective HMBC correlations of clausenaexcavin (**1**)

EXPERIMENTAL

GENERAL

Optical rotations were measured on a JASCO P-1020 digital polarimeter. UV and IR spectra were recorded on SPECORD S100 (Analytikjena) and Perkin–Elmer FTS FT-IR spectrophotometers, respectively. The ^{1}H– and ^{13}C–NMR spectra were recorded using 400 MHz Bruker FTNMR Ultra Shield and 500 MHz Varian UNITY INOVA spectrometers. Chemical shifts were recorded in parts per million (δ) in $CDCl_3$ with tetramethylsilane (TMS) as an internal reference. High resolution mass spectrum was obtained using MAT 95 XL mass spectrometer. Quick column chromatography (QCC) and column chromatography (CC) were carried out on silica gel 60 H (Merck, 5–40 μm) and silica gel 100 (Merck, 63–200 μm), respectively. Precoated plates of silica gel 60 F_{254} were used for analytical purposes.

PLANT MATERIAL

The dried fruits and stems of *C. excavata* were collected from Satoon Province, southern part of Thailand in May 2008. Botanical identification was achieved through comparison with a voucher specimen number QBG 6277 in the herbarium collection of Queen Sirikit Garden, Mae Rim District, Chiang Mai, Thailand.

EXTRACTION AND ISOLATION

The fruits of *C. excavata* (250 g) were extracted with hexane and CH_2Cl_2, respectively, over a period of 3 days each at rt. The hexane and CH_2Cl_2 extracts were combined (987.7 mg) and chromatographed by CC over silica gel eluted with a gradient of EtOAc-hexane (20% EtOAc-hexane to 100% MeOH) to give twenty-two fractions (A-V). Fraction I (168.0 mg) was separated by Sephadex

LH-20 with 60% CH_2Cl_2-MeOH to provide five subfractions (I1-I5). Subfraction I3 (85 mg) was purified by CC using 8% $CHCl_3$-hexane to afford compound **1** (4.2 mg, 0.42 %). Fraction T (384.8 mg) was also separated by Sephadex LH-20 eluting with 60% CH_2Cl_2-MeOH to obtain four subfractions (T1-T4). Compound **2** (1.5 mg, 0.15 %) and compound **3** (4.2 mg, 0.42 %) were derived from subfraction T1 (173.4 mg) by CC with 3% acetone-CH_2Cl_2.

The stems of *C. excavata* (3.20 kg) were extracted with EtOAc, over a period of 3 days each at rt. The EtOAc extract (70.50 g) was subjected to quick column chromatography (QCC) over silica gel and eluted with a gradient of acetone-hexane (100% hexane to 100% acetone) to give twenty-five fractions (A-Y). Fraction I (297.3 mg) was further purified by QCC with 27% CH_2Cl_2-hexane provided twelve subfractions (I1-I12). Solids of subfraction I4 (27.0 mg) were washed with hexane to give compound **4** (4.4 mg, 0.000062 %). Fraction N (387.4 mg) was separated by QCC with 45% CH_2Cl_2-hexane to afford nine subfractions (N1-N9). Compound **5** (12.5 mg, 0.00177 %) was obtained from subfraction N4 by CC with 12% acetone-hexane whereas compound **6** (21.5 mg, 0.00305 %) was derived from solids of subfraction N9 (75.0 mg) by washing with CH_2Cl_2.

Clausenaexcavin (**1**): Colorless viscous oil. $[\alpha]_D^{29}$ -223.4° (*c* 0.04, $CHCl_3$).UV λ_{max} ($CHCl_3$) (log ε): 207 (2.26), 230 (2.27), 258 (2.30), 318 (2.47) nm. IR (neat) ν_{max}: 3408, 2971, 2932, 1718, 1612 cm^{-1}. ^{1}H–NMR (500 MHz, $CDCl_3$): 7.60 (1H, d, *J* = 9.5 Hz, H-4), 6.93 (1H, d, *J* = 8.5 Hz, H-5), 6.80 (1H, d, *J* = 8.5, H-6), 6.25 (1H, d, *J* = 9.5 Hz, H-3), 5.77 (1H, d, *J* = 16.0 Hz, H-6′), 5.73 (1H, m, H-5′), 4.99 (1H, dd, *J* = 3, 11.5 Hz, H-1′a), 4.09 (1H, dd, *J* = 9, 11.5 Hz, H-1′b), 3.98 (1H, dd, *J* = 3.0, 9.0 Hz, H-2′), 2.44 (1H, dd, *J* = 6.0, 14.0 Hz, H-4′a), 2.29 (1H, dd, *J* = 7.5, 14.0 Hz, H-4′b), 1.34 (3H, s, H-9′), 1.30 (3H, s, H-8′) and 1.29 (3H, s, H-10′). ^{13}C–NMR (125 MHz, $CDCl_3$): 160.7 (C-2), 146.4 (C-7), 143.9 (C-4), 143.7 (C-8a), 140.3 (C-5′), 131.5 (C-8), 120.3 (C-6′), 119.5 (C-5), 113.5 (C-3), 113.4 (C-4a), 113.3 (C-6), 77.9 (C-2′), 72.9 (C-3′), 72.3 (C-7′), 65.1 (C-1′), 41.5 (C-4′), 29.9 (C-8′ and C-10′), 22.8 (C-9′). EIMS *m/z* (% intensity) 346 (M^+-H_2O, 10), 331 (75), 247 (100), 204 (89), 175 (53). HREIMS *m/z* 346.1422 $[M-H_2O]^+$ (calcd. for $C_{19}H_{22}O_6$, 346.1416).

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